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Repair Strength Dependence on Solder Protein Concentration: A Study in Laser Tissue-Welding

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Background and Objective: **A novel laser-activated solid solder has been coupled with a diode laser to investigate the dependence of the solder protein concentration on the tensile strength of the soldered tissues. The uncertainty of laser welding, due to the fluid glue, was overcome using the solid solder.**
Study Design/Materials and Methods: **Sixty-two severed rat tibial nerves and vas deferens were repaired using rectangular protein bands with two different albumin concentrations (58% and 68% by weight). The laser power (90 mW and 140 mW), dose (12.9 ± 0.7 J/mg, mean ± s.d.), and solder dimensions (thickness = 0.15 ± 0.01 mm, surface area = 7.8 ± 0.4 mm^2) were kept constant during the operations.**
Results: **The laser welds with high protein solder concentration were significantly ($P < 0.05$) stronger (28 ± 3.5 g) than the welds with low protein solder concentration (23 ± 5 g).**
Conclusions: **The average tensile strength of the laser soldered tissues increased as the protein solder concentration increased.**
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Key words: dye; dose; surface area; nerve; vas deferens

INTRODUCTION

The binding mechanism between the solder and the tissue during the laser irradiation is still unknown. Nevertheless, the majority of researchers agree that the proteins contained in the solder (collagen, albumin, fibrin) and in the tissue (mostly collagen) play a primary role in laser tissue welding [1–5]. The nature of this protein bond is currently in dispute; indeed some researchers believe that the bond is due to protein crosslinking and others support the noncovalent nature of the bond [6,7]. The role of the protein concentration in increasing the weld tensile strength has been investigated previously, but the use of fluid glues in laser tissue repair prevented a quantitative analysis [8,9]. Indeed, fluid glues are applied on the tissue without a constant thickness and surface area, which influence the heat penetration through the solder and the tensile strength of the solder weld. In this study, a new albumin-based solder was used in conjunction with a Diode laser [10,11]. The solder is a solid malleable paste that can be molded into a variety of shapes (hollow tubes and rectangular bands, e.g.) with precise dimensions. The tensile strength of the solder repair has been investigated by varying the concentration of protein rectangular bands, which were bonded to the tissue with the laser. The dimensions of the bands were finely controlled, overcoming the uncertainty related to the fluid glues.

MATERIALS AND METHODS

Thirty-two adult male Wistar rats weighing ~500 g were used in this study. Approval and con-

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sent for the study were obtained from our institution's Research on Animal Ethical Review Committees.

Laser System

A GaA1As Diode laser (SDL, Ca) coupled with an optical fibre was used in this study. The laser wavelength was 810 nm (2 nm bandwidth) and the laser power ranged from 90 mW to 140 mW. The multimode fibre had a core diameter of 100 μm, numerical aperture of 0.3, and the laser spot size on the solder was ~200 μm. An external current driver sent continuously 1–3 seconds long gating pulses to the laser and the pulse number was chosen by the operator to achieve the required laser exposure dose (energy/solder weight).

Protein Solder

The solid solder contained distilled water, Bovine Serum Albumin (BSA, Sigma, St. Louis, MO) in two concentrations (58% ± 1%, 68% ± 1% by weight) and Indocyanine Green (I.G., 0.25% ± 0.01% by weight) to absorb the infrared radiation [12]. A parallel plate vice pressed the solder and its thickness was reduced to 0.15 ± 0.01 mm. The protein sheet was then cut with a scalpel into rectangular bands and accurately weighted with an analytical balance (Mettler H20, s.d. = 0.03 mg) or an electrobalance (Cahn 29, s.d. = 1 μg). The band surface area was calculated with the formula $S = P_b \cdot (hd)^{-1}$, where P_b is the weight of the band, *h* its thickness, and *d* the band density. The protein solder had an absorption peak at 805 nm, corresponding to ~7.5 μm absorption length [13]. For storage, the protein bands were positioned between thin metallic foils to preserve their flat shape and to shield them from light and then were stored in the refrigerator at 5°C. The bands were composed of sterile compounds, which were handled and mixed using sterile instruments. No further sterilization was carried out on the bands.

Error and Statistical Analysis

The standard deviation of the solder surface area was calculated by the propagating error formula: $[\sigma(S)]^2/S^2 = [\sigma(P_b)]^2/P_b^2 + [\sigma(d)]^2/d^2 + [\sigma(h)]^2/h^2$, where σ = standard deviation, S = band surface area, P_b = band protein weight, d = solder density, h = band thickness. Statistical comparison of mean values was made using the Student's t-test for unpaired observations (0.05 level of significance). The linear regression and histogram were generated by Sigma Plot (2.01 version).

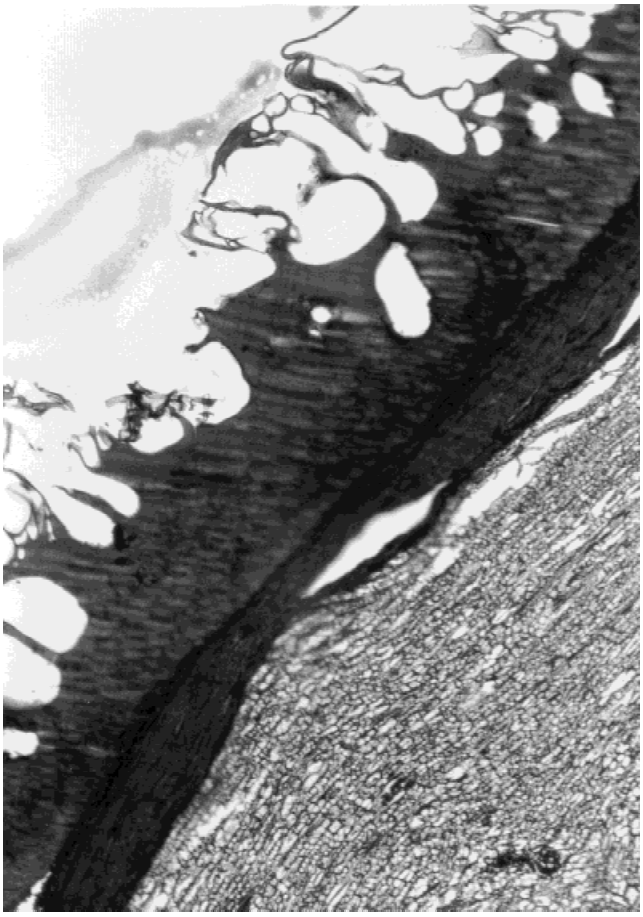

Fig. 1. Distal longitudinal neurorraphy immediately after laser-solder repair. The laser-induced heat has denatured the BSA band, which is welded with the perineurium (top right). Nevertheless, the axons underneath appear undamaged (×100 Masson's Trichrome).

Laser-Solder Repair

Animals, already used for other experiments, were anaesthetized with an Halothane/O_2 mixture and sacrificed by an intracardiac injection of pentabarbitone sodium. An operating microscope was used throughout the operations. The rats had the tibial nerves or the vas deferens exposed. These tissues were operated on using the laser in conjunction with the protein bands [11]. The laser-solder technique involved initial aligning of both stumps of the severed tissue with microforceps. A protein band was then positioned longitudinally across the repair site using microforceps. The laser beam was passed over the length of the band and welded it to the perineurium, forming the bond (Fig. 1). During laser ir-

Fig. 2. The operation site immediately after the laser-solder repair. Four protein bands hold together the nerve stumps after the laser irradiation (×30).

radiation, the beam spot was visible because the solder turned brown on a single pass signaling denaturation. This change in color allowed the surgeon to direct the beam to the solder steadily and without significant energy loss. Rotation of the nerve was obtained by gently pulling the gauze previously positioned underneath the nerve, so that one to three other bands could be applied, each ~90° apart (Fig. 2). Vas deferens were repaired with only two protein bands and without tissue rotation to assess the impact of the rotational stress on the soldered tissue strength. The animals were divided into five groups and the repairs were performed using the sets of parameters displayed in Table 1. Groups 1–4 were established to investigate the tensile strength of the soldered tissues by varying the BSA concentration. In these groups, the total solder surface area and laser parameters were kept constant. The dependence of the solder weld strength on the protein band surface area was investigated in group 5. Protein bands with four different total surface areas were applied to the nerves (Table 2).

In all five groups, the protein band thickness was constant (0.15 ± 0.01 mm) as the heat penetration and consequently the weld strength depend on it [14].

Tensile Strength Measurements

Sections (~1 cm long) of the repaired tissues were harvested immediately after the operation and they were used for tensile strength test. A metal foil strain gauge (FT30C, Grass Inst. Quincy, MA) was used to measure the breaking force of the laser-solder weld [11].

RESULTS

The breaking forces of groups 1 and 3, with higher BSA concentration, were 28 ± 3.4 g and 27.2 ± 3.6 g, respectively. In groups 2 and 4 (lower BSA content), the breaking forces averaged 22.9 ± 4.2 g and 22.7 ± 5.5 g, respectively (Fig. 3). These values were significantly different ($P < 0.05$). In group 5, a linear relation ($r^2 = 0.99$) was found between the solder breaking force and the solder surface area when band thickness, laser power, and dose are unvaried (Fig. 4). The slope of the line was 3.5 g/mm^2, and it corresponded to the breaking force increment for each mm^2 of added solder (Table 2).

The repaired tissue suffered two kind of failure when it was pulled apart. Of the bands with higher protein concentration, 53% broke in two where the tissue ends meet (volume rupture), whereas the remaining 47% detached from one of the two tissue ends and remained attached to the other side without breaking into two parts (interface rupture). Of the bands with lower protein concentration, 62% suffered interface ruptures, and the remaining 38% failed because of volume ruptures.

DISCUSSION

The tensile strength of the laser weld increased with the protein concentration if the radiation power, dose, and solder dimensions (thickness and surface area) were kept constant. This result is in agreement with the better performances in tissue welding of dense fluid glues (45–50% BSA concentration) than low concentration fluid glues [4,10,12,13,15,16]. The tensile strength of the laser repair was not related precisely to the solder protein concentration in pre-

TABLE 1. Operating Groups*

	BSA% (g/g)	Repaired tissue	Laser power (mW)	Irradiation time (s)	Laser dose (J/mg)	Solder area (mm^2)
Group 1	68	Tibial nerve n = 10	90 ± 3	198 ± 16	13.6 ± 0.7	7.81 ± 0.67 (4 bands)
Group 2	58	Tibial nerve n = 10	90 ± 3	203 ± 19	13.7 ± 0.7	7.77 ± 0.67 (4 bands)
Group 3	68	Vas deferens n = 11	140 ± 4	113 ± 14	12.3 ± 1.0	7.69 ± 0.60 (2 bands)
Group 4	58	Vas deferens n = 11	140 ± 4	114 ± 12	12.0 ± 1.1	7.78 ± 0.62 (2 bands)
Group 5	68	tibial nerve n = 20	90 ± 3	37–210	13.6 ± 1.5	1.45–8.23 (1–4 bands)

*Mean values ± standard deviations of the solder and laser parameters are given for the five animal groups. The total surface area and laser dose of the protein bands were kept constant during the operations. The approximated surface dimensions of each protein band were 0.5 × 3.5 mm (groups 1, 2, 5) and 0.6 × 6.5 mm (groups 3, 4). One to four protein bands were applied to the tibial nerves of group 5.

TABLE 2. Solder Tensile Strength*

Laser power (mW)	Solder surface area (mm^2)	Laser dose (J/mg)	Laser exposure time (s)	Laser weld strength (g)
90 ± 3	1.45 ± 0.28	13.7 ± 2.5	37 ± 6	5.8 ± 1.5
90 ± 3	2.97 ± 0.35	13.6 ± 2.0	75 ± 7	13.3 ± 2.7
90 ± 3	4.70 ± 0.44	13.4 ± 0.9	117 ± 8	18.6 ± 2.7
90 ± 3	8.23 ± 0.70	13.8 ± 0.7	209 ± 11	30.4 ± 4.1

*Protein bands with four different total surface areas were applied to the nerves of group 5. Five nerves were operated on for each solder surface area. The laser parameters (mean ± s.d.) and tensile strength of the soldered nerves are also indicated.

vious studies, because the surgeon was unable to control the required surface area and thickness of the fluid glue on the tissue.

The failure of the laser-solder welding was due to a detachment of the protein bands from the tissue (interface rupture), or to a central cut of the bands in two parts (volume rupture). In the first case, the BSA-collagen bonds of the solder and tissue interface were broken [2]. However, in volume ruptures the intermolecular BSA crosslinks might be cut. An increase in BSA concentration may suggest the formation of a higher number of protein bonds, which results in an increased laser-solder repair strength. In particular, the increase of BSA concentration (68%) seemed to enhance the solder-perineurium interface bonds, which might be responsible for the lower rate of interface ruptures. The correct dimensions of the bands should balance interface and volume ruptures to avoid laser-solder repair failure.

The laser power used to form a strong solder tissue weld (~27 g) was significantly higher for vas deferens anastomosis (140 mW) than for tibial nerve repair (90 mW), when the radiation doses

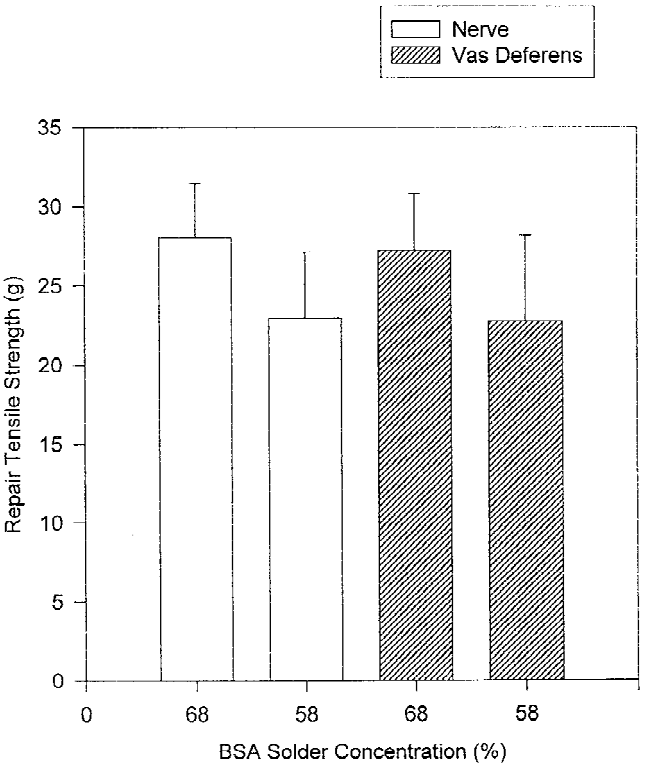

Fig. 3. Acute tensile strength (mean ± s.d.) of the laser welded nerves and vas deferens versus BSA solder concentration for groups 1–4.

were comparable. Indeed, previous trials in our laboratory indicated that the vas deferens anastomosis tensile strength was very weak if the laser power was kept at 90 mW during operation. The different tissue composition and structure of the perineurium and the adventitia of the vas deferens might be responsible for the different power level required for an efficient solder-tissue weld. Also, the protein bands, applied on the vas defer-

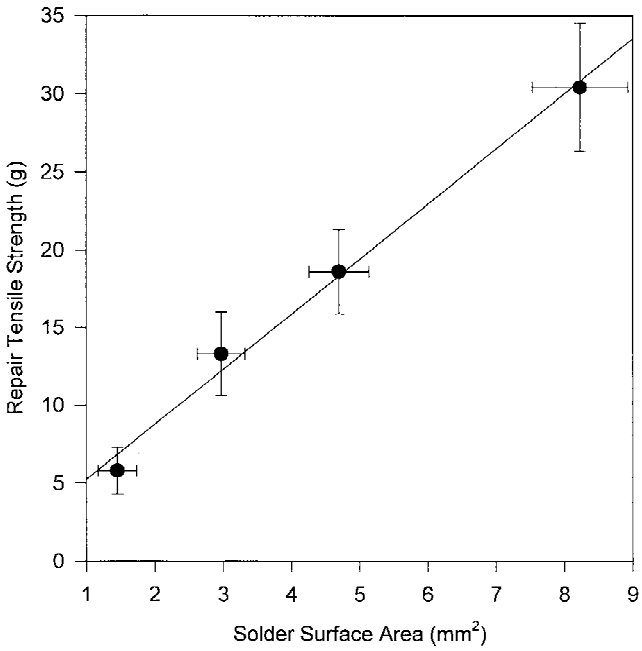

Fig. 4. Acute tensile strength (mean ± s.d.) of the laser soldered repair vs. the surface area of the bands (applied on the nerves) for group 5.

ens, were twice as big as those applied on the nerves; therefore, a higher laser power might be required to increase the heat penetration through the larger volume of the bands.

The solder weld strength was proportional to the surface area of the protein solder applied to the repair site if the radiation dose, thickness, and surface area of the protein bands were constant. Therefore, the tensile strength of a laser-solder repair could be determined with sufficient precision, avoiding weak nerve coaptation and its consequent rupture. It is remarkable to note that 1 cm^2 of solder corresponds theoretically to ~350 g of tensile strength. Nevertheless, the heat diffusion can be less efficient through large bands than small bands when the same laser power and dose are used. There is a limitation in increasing the solder dimensions because the interface welds of big bands can be weaker than the interface welds of small bands.

The large error of the weld breaking force (s.d. 4 g) may be due to the asymmetric distribution of the pulling forces acting on the bands, which can be overloaded and more subject to failure. The dye distribution in the solder also was not perfectly uniform (due to the high BSA content) despite the finely measured concentration of the dye. As a consequence, the laser penetration and heat diffusion inside the solder could vary locally, contributing to the standard deviation of the repair strength. The mechanical stress on the solder repair site, due to the tissue rotation, appeared not to be significant (s.d. ~4 g for both the rotated nerves and for the not-rotated vas deferens) if particular care was used during the surgical procedure.

In conclusion, the higher protein concentration in the solder resulted in a stronger laser repair. The protein band dimensions and laser radiation dose could be optimized, ensuring an adequate solder strength and avoiding the failure of the repaired tissue. Lack of knowledge about the binding mechanism between the protein solder and the tissue leaves us unable to explain the power variation that occurred for an efficient laser soldered weld of different tissues. For this reason, the "measurable" solid solder will be useful to test solder-tissue binding models in future experiments.

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